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Original Article

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Uterine involution and progesterone level during the postpartum period in Barbary ewes in north Libya

M.S. Medan[1,2,*] and T. EL-Daek[1]
[1]*Department of Theriogenology, Faculty of Veterinary Medicine, Omar AL-Mukhtar University, AL-Bayda, Libya*
[2]*Department of Theriogenology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt*

Abstract
The objectives of the present study were to determine the time of uterine involution and ovarian activity using ultrasound examination and progesterone assay. Weekly progesterone levels were measured starting one week postpartum until two weeks after the 1st postpartum estrus in Barbary ewes lambed during winter in AL-Bayda city, north of Libya. A total of 15 Barbary ewes were used in the present study distributed in three groups according to the month of lambing as group 1 (lambed in January), group 2 (lambed in February) and group 3 (lambed in March). Ewes were examined weekly by trans-rectal ultrasound to check involution of the uterus starting one week after lambing until complete uterine involution. Blood samples were collected from the jugular vein, and serum was separated and stored at -20 ºC until measuring progesterone using ELISA. Results showed that uterine involution completed at day 35 postpartum in groups 1 and 2, while it occurred at day 28 in group 3. The mean progesterone level was basal (less than 1 ng/ml) for a long period and started to increase at days 119, 99 and 77 postpartum in group 1, 2 and 3, respectively. One ewe did not show estrus at all during the period of study in group 2 and there were no growing follicles on their ovaries. The obtained results indicate that, uterine involution as determined by ultrasound completed earlier in ewes lambed in March than those lambed in February or January. Also, progesterone level and ultrasound examination showed that there was no ovarian activity for a longtime after parturition indicating that reproduction in Barbary ewes tends to be seasonal in AL-Bayda city, north Libya.
Keywords: Ewes, Ovarian activity, Postpartum uterine involution, Ultrasound.

Introduction

The postpartum period is characterized by uterine involution and restoration of ovarian functions, since both should occur to establish a new pregnancy. The completion of uterine involution was defined as the day when the diameter of the uterus returned to the original non-pregnant size as observed during the normal estrous cycle (Takayama *et al.*, 2010).
Uterine involution occurs in a decreasing logarithmic scale with the greatest change occurring during the first few days after parturition (Noakes *et al*., 2009). Completion of uterine involution and resumption of sexual activity following parturition in ruminants normally depend on several factors such as nutrition, nursing of offspring and season of parturition (Delgadillo *et al*., 1998; Yavas and Walton, 2000).
Previously, techniques such hormone measurements (Ishwar, 1995), radiography (Kene, 1991; Tian and Noakes, 1991) and laparotomy (Ishwar, 1995; Rubianes *et al*., 1996) were used to study the dynamics of uterine involution in small ruminants. However, these techniques are not practical under field conditions (Goddard, 1995).
The use of B-mode trans-rectal ultrasonography for imaging the reproductive tract provides real time, functional and clinical information such the number of follicles (Vinoles *et al*., 2004), pregnancy (Medan and Abd El-Aty, 2010) and uterine pathology (Yilmaz *et al*., 2008). Moreover, trans-rectal ultrasonography can be used under field conditions to determine uterine involution in different animals (Lohan *et al*., 2004; Hajurka *et al*., 2005 and Yilmaz and Ucar, 2012). Ultrasonography plays a key role to differentiate the normal or abnormal postpartum uterus and in early diagnosis of any abnormal condition related to uterus (Feldman and Nelson, 1996).
In sheep, the interval between parturition and the resumption of ovarian activity has been shown to be influenced by some factors such as suckling intensity (Schirar *et al*., 1989) or season of parturition (Pope *et al*., 1989; Delgadillo *et al*., 1998). Pope *et al*. (1989) reported that there are large breed differences on the extent of the postpartum anestrus prior to a fertile estrus, reflecting a strong genetic component in addition to seasonal influences.
The aim of the present study was to determine the time required for uterine involution in Barbary ewes by mean of ultrasonography and start of ovarian activity through progesterone measurement.

Materials and Methods

This study was carried out on 15 pluriparous Barbary ewes at AL-Bayda city, north Libya (latitude 21.22 N

***Corresponding Author:** Dr. Mohamed Sabry Medan. Current Address: Department of Theriogenology, Faculty of Veterinary Medicine, Omar AL-Mukhtar University, AL-Bayda, Libya. Email: medan69@hotmail.com

and longitude 32 E). The selected ewes had normal lambing during winter and giving birth to singleton. Ewes were distributed on 3 groups according to the date of lambing as group 1 (n=5; lambed in January), group 2 (n=5; lambed in February) and group 3 (n=5; lambed in March). Age of the animals ranged between 4 to 6 years and their body weight ranged between 45-55 kg. Each ewe had fed on 1.5 kg concentrates, divided into two times daily beside roughage. Water was available ad libitum and mineral salt licks were also available during the whole period of the study.

Estrus behavior was monitored two times a day starting one week after parturition up to the end of study, using an intact active ram. A female was recorded as being in estrus when she accepted mounting attempt by the male.

Examination of the postpartum uterus:

Uterine involution was checked through trans-rectal ultrasound examination weekly starting from one week after delivery through complete uterine involution and continued until start of ovarian activity using B-mode ultrasound machine (Landwind, C30 Vet) equipped with a multifrequency 5-10 MHz probe. For ultrasonic examination, the ewe was restrained in a standing position with the help of an assistant. The rectum was evacuated from feces and air with the aid of the lubricated fingers. Thereafter, the lubricated transducer (fixed to an extension rod) was introduced into the rectum. The transducer was moved medially and laterally to get the best view of the uterine horn and maximum diameter was recorded. Uterine involution was considered to be complete when there was no further reduction in the uterine diameter for two successive examinations (Zdunczyk *et al*., 2004).

Blood sampling and hormonal assay:

Blood samples (10 ml) were collected weekly from each ewe from the jugular vein into vacutainer tubes without anticoagulant, starting one week after lambing until 2 weeks after the appearance of the first postpartum estrus. The tubes were centrifuged at 3000 rpm for 15 minutes. Blood serum was separated and deep-frozen at -20 ºC until assessment of progesterone concentration using Enzyme-linked Immunosorbent Assay (ELISA) method.

Statistical analysis:

Results were expressed as means ± SE (standard errors). The analysis of variance (ANOVA) was used to test the significance of differences between means. Significance was assigned at $P<0.05$. Duncan's multiple range test was applied for post hoc comparison using SPSS software, (SPSS, version 21, 2012).

Results

Uterine involution:

Postpartum gravid horn diameter, as estimated by trans-rectal ultrasonography in the three groups was shown in Table 1. Uterine involution occurred at day 35 postpartum in group 1 and 2, while it occurred at day 28 postpartum in group 3. The results showed that uterine involution in the third group is completed in a shorter time compared with the first group and second group. Uterine horn diameter decreased sharply ($P<0.05$) in all groups within the first 4 weeks after parturition.

Table 1. Mean (± SEM) of postpartum uterine horn diameters as determined by trans-rectal ultrasonography in ewes lambed in January, February and March.

Day of examination	Uterine horn diameter (cm)		
	Group 1 (n=5) January lambing	Group 2 (n=5) February lambing	Group 3 (n=5) March lambing
7	5.62 0.38[a]	5.28 ± 0.32[a]	5.24 ± 0.37[a]
14	4.44 ± 0.37[b]	3.92 ± 0.24[b]	3.86 ± 0.35[b]
21	3.42 ± 0.29[c]	3.06 ± 0.22[c]	2.84 ± 0.30[c]
28	2.68 ± 0.17[d]	2.36 ± 0.20[d]	1.98 ± 0.17[d]
35	2.10 ± 0.07[de]	2.04 ± 0.15[de]	1.78 ± 0.09[d]
42	1.80 ± 0.06[e]	1.88 ± 0.14[de]	1.74 ± 0.12[d]
49	1.78 ± 0.07[e]	1.76 ± 0.06[e]	1.70 ± 0.09[d]
56	1.78 ± 0.07[e]	1.76 ± 0.07[e]	1.70 ± 0.11[d]
63	1.74 ± 0.05 [e]	1.76 ± 0.08 [e]	1.68 ± 0.10[d]

Values with different superscripts in the same column are significantly different ($P<0.05$).

Ultrasonography of the postpartum ovaries:

Trans-rectal ultrasound examination of postpartum ovaries in the present study did not show any follicle > 3 mm in diameter for about 25 days postpartum in all animals. Small follicles (size 3- 5 mm in diameter) were recorded on the ovaries after 25 days postpartum, while follicles > 5 mm in diameter were detected after 50 days postpartum (data not shown). On the other hand, ovulation and corpora lutea formation detected only after estrus.

Progesterone concentration during the postpartum period:

The mean progesterone level in studied groups remained basal (< 1 ng/ml) for a long period after lambing as shown in Fig. 1. Progesterone levels started to elevate at day 119 postpartum in group 1. In group 2, progesterone levels elevated at day 99 postpartum, while in group 3, it started to increase at day 77 postpartum. Figs. 2, 3 and 4 show progesterone level in the individual ewes in groups 1, 2 and 3, respectively. As shown in Fig. 2, one ewe showed an elevated progesterone level at day 91 postpartum without showing estrus. In addition, progesterone level was basal during the whole period of study in one ewe in group 2. The ovaries of that ewe were smooth and did not shown any growing follicles as determined by trans-rectal ultrasonography and also it did not show estrus.

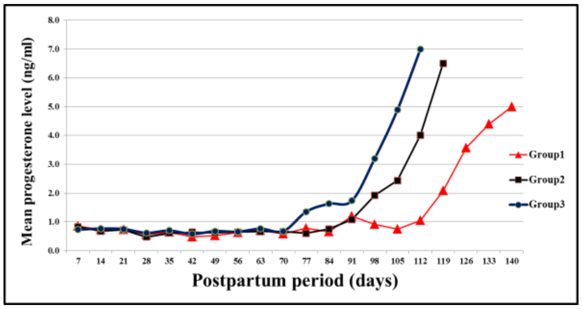

Fig. 1. Mean progesterone levels in postpartum ewes lambed in January (Group 1); February (Group 2); March (Group 3).

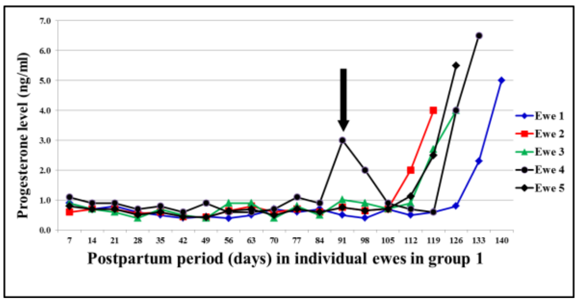

Fig. 2. Progesterone level in individual ewes lambed in January (Group 1). Arrow indicates a rise in progesterone level in one ewe without showing estrus before elevation.

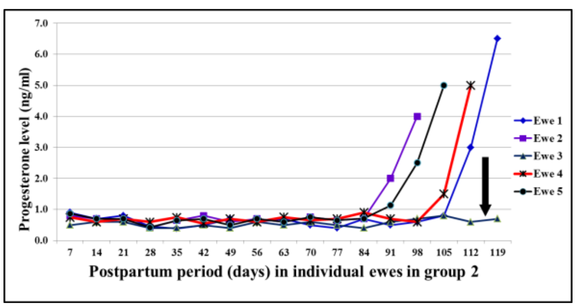

Fig. 3. Progesterone level in individual ewes lambed in February (Group 2). Arrow indicates one ewe that did not show estrus at all.

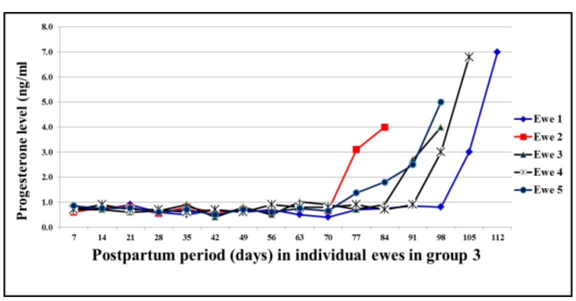

Fig. 4. Progesterone level in individual ewes lambed in March (Group 3).

As shown in Table 2, the first postpartum ovulation and formation of corpora lutea as determined by elevated progesterone (> 1 ng/ml) level occurred at day 117.00 ± 3.73, 94.25 ± 4.49 and 78.60 ± 4.28 in groups 1, 2 and 3, respectively. The period from parturition until estrus and ovulation was significantly longer in group 1 (lambed in January) than group 2 (lambed in February) and group 3 (lambed in March), however, ovulation rate was nearly similar (Table 2).

Table 2. The day of postpartum estrus and ovulation rate in ewes lambed in January (group1), February (group 2) and March (group 3).

Groups	Day of postpartum estrus	Ovulation rate
Group 1	117.00 ± 3.73[a]	1.4
Group 2	94.25 ± 4.49[b]	1.5
Group 3	78.60 ± 4.28[b]	1.4

Values with different superscripts in the same column are significantly different (P<0.05).

Discussion

There are no data or literature information about postpartum uterine involution in Barbary ewes in Libya and so, the present study is considered very important and necessary to provide information that can be useful for improving reproductive efficiency. The ability to achieve maximum reproductive efficiency in ewes depends upon understanding postpartum changes of uterus and ovaries (Lewis and Blot, 1983).

The uterus experiences considerable distension and distortion of tissues and intensive glandular development to accommodate and nourish the developing fetus to term. The uterus must undergo gross anatomical changes together with extensive remodeling and changes in tissue mass and function during the postpartum period before rebreeding and pregnancy can be established (Hunter, 1980; Sanchez *et al*., 2002).

The present study on Barbary ewes lambed in January, February and March indicated that uterine involution occurred mostly between day 28 and 35 after parturition. This is in agreement with the results reported in sheep by O'Shea and Wright (1984) and Zdunczyk *et al*. (2004). Van Niekerk (1976) recorded that uterine involution and regeneration of the epithelium in ewes may not be completed until day 26 during the breeding season, while during anestrous involution was delayed until day 30.

Another study demonstrated that uterine involution was completed within 21 days postpartum in sheep (Fernandes *et al*., 2013). Tian and Noakes (1991), using radio-opaque markers, recorded that uterine involution was completed around day 29 after lambing. The results of the present study showed that uterine involution in the third group is completed in a shorter time compared with the first group and second group indicating that the date of lambing affects uterine involution. A previous study on sheep showed that uterine involution was completed in 35 days postpartum in the majority of examined cases (Zdunczyk *et al*., 2004). However, Hauser and Bostedt (2002) mentioned that uterine involution in sheep was delayed in cases of dystocia and cesarean section and retention of placenta. Puerperal complications such as retained placenta and/or acute puerperal endometritis to metritis usually delay uterine involution (Hajurka *et al*., 2005). In sheep, there is considerable variation in the annual anestrous season depending on the geographical region and day length. Sexual activity in sheep is primarily controlled by the ratio of daylight to dark. In temperate regions, estrus becomes more frequent as the daylight becomes shorter. Longer daylight decreases gonadotropin secretion and causes ovarian cycles to cease.

In contrast, a shift from long to short daylight results in increased gonadotropin secretion and onset of ovarian cycles. The reason is that photoperiod is transduced to a signal influencing gonadotropin-releasing hormone (GnRH) release and communicate directly with GnRH neurons in the hypothalamus (Hileman *et al*., 2011). In the present study, the mean interval from parturition to the resumption of the ovarian activity differed according to the month of lambing. It was 117.00±3.73 days in ewes lambed in January, which is considered significantly longer than those lambed in February (94.25±4.49 days) and March (78.60±4.28 days).

These results indicate that the time of the year is a major factor controlling the duration of postpartum ovarian activity, which is in agreement with results obtained in ewes by other workers (Santiago-Moreno *et al.*, 2000; Hileman *et al*., 2011).

Blood concentration of progesterone is a good indicator of luteal function during the postpartum period since progesterone is the major steroid synthesized by the corpus luteum. It is well known that systemic progesterone concentrations greater than 1 ng/ml are associated with presence of a corpus luteum or a luteinized follicle (Berardinelli *et al*., 2001). The interval from parturition until the first ovulation in ewes is greatly influenced by season (Hileman *et al.*, 2011). The postpartum anestrous interval is longest when ewes lamb during the winter as length of the photoperiod is increasing. The interval is shorter when lambs are born during the summer (Wettemaan, 1980). Moreover, there are large breed differences on the extent of the postpartum anestrus prior to a fertile estrus, reflecting a strong genetic component in addition to seasonal influences (Pope *et al*., 1989). In the present study, all examined ewes showed a state of ovarian inactivity during the first 70 days after parturition, as indicated by the low serum progesterone level. Prolonged postpartum luteal activity might be a result of the high prolactin hormone observed in the first few weeks postpartum (Lamming *et al*., 1974). Also, delayed onset of the ovarian activity might be due to a negative energy or protein balance. Ewes nursing lambs were often in negative energy balance during the first month of lactation (Robinson *et al*., 1979).

One ewe in the group 1 showed a higher progesterone level at day 91 postpartum without showing estrus before that elevation or follicular activity as determined by ultrasound. This elevation may be due to luteinization of small follicles which cannot be detected by ultrasonography. Moreover, one ewe in group 2 did not show estrus at all or follicular growth as determined by ultrasound. This is also confirmed by its basal progesterone level during the period of study. The results of the present study demonstrate that the duration of postpartum anestrus in Barbary ewes is influenced by the time of the year when parturition occurs. In addition, it appeared that ovarian activity in Barbary ewes tends to be seasonal since ewes lambed in January, February and March started ovarian activity at the start of the following breeding season.

Conclusion

The obtained results indicate that, uterine involution as determined by ultrasound was completed earlier in ewes lambed in March than those lambed in February or January. Also, progesterone level and ultrasound examination showed that there was no ovarian activity for a long time after parturition indicating that reproduction in Barbary ewes tends to be seasonal in AL-Bayda city, north Libya.

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